



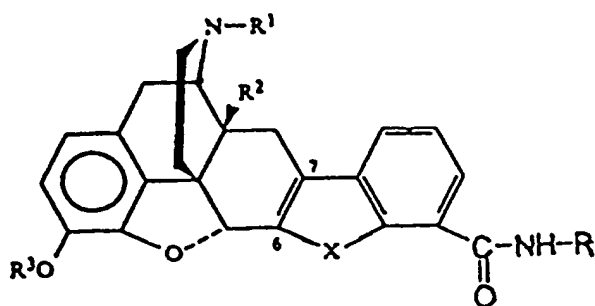
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(54) Title: **DELTA OPIOID RECEPTOR ANTAGONISTS**



(57) Abstract

Compounds are provided of formula (1), wherein R¹ is (C₁-C₅)alkyl, C₃-C₆(cycloalkyl)alkyl, C₃-C₇(cycloalkenyl)alkyl, (C₆-C₁₂)aryl, (C₆-C₁₂)aralkyl, trans-(C₄-C₅)alkenyl, allyl or furan-2-ylalkyl, R² is H, OH or O₂C(C₁-C₃)alkyl; R³ is H, (C₆-C₁₀)aralkyl, (C₁-C₅)alkyl or (C₁-C₅)alkylCO; X is O, S or NY, wherein Y is H, benzyl or (C₁-C₃)alkyl; R is CH(Z)CO₂Y, wherein Z is H, CH₂CO₂Y or (CH₂)_nN(R⁴)(R⁵), wherein n is 1-5 and R⁴ and R⁵ are individually H, (C₁-C₄)alkyl, phenyl, (C=NH)NH₂, benzyloxycarbonyl or (C=NHNO₂); and the pharmaceutically acceptable salts thereof.

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DELTA OPIOID RECEPTOR ANTAGONISTS

5 **Background of the Invention**

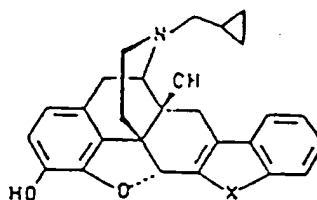
Endogenous opioid peptides are involved in the mediation or modulation of a variety of mammalian physiological processes, many of which are mimicked by opiates or other non-endogenous opioid ligands. Endogenous opioid peptides and opiates mediate their pharmacologic effects
10 via interaction with opioid receptors both in the central nervous system (CNS) and peripheral nervous systems. See J. H. Jaffe et al., Opioid Analgesics and Antagonists, in The Pharmacological Basis of Therapeutics, A. G. Gilman et al., eds., Pergamon Press, NY (1990) at pages 485-521. The principal target tissues in the central nervous system are the brain and the spinal cord. The
15 major sites in the peripheral nervous system are the gastrointestinal tract, lymphocytes, and a variety of other tissues. The constipating action and inhibitory modulation of the immune response by morphine are examples.

D. R. Brown et al., Neuropharmacol., **24**, 181 (1986) employed quaternized opiates as pharmacologic tools in an attempt to isolate peripheral
20 from central effects. However, these compounds exhibited considerably lower affinity for opioid receptors than their tertiary amine precursors. In an attempt to circumvent this problem, β -naltrexone and β -oxymorphanine incorporating hydrophilic groups attached to the C-6 position of the morphinan system were synthesized by S. Botros et al., J. Med. Chem., **32**, 2068 (1989). Botros et al.
25 reported that such compounds, particularly opiates with zwitterionic moieties, exhibited substantially reduced access to the CNS without substantially decreasing receptor activity.

The fact that the effects of endogenous and exogenous opioids are mediated by at least three different types [μ (μ), delta (δ), kappa (κ)] of
30 opioid receptors raises the possibility that highly selective exogenous opioid agonist or antagonist ligands might have therapeutic applications. See W.R. Martin, Pharmacol. Rev., **35**, 283 (1983). Thus, if a ligand acts at a single

opioid receptor type or subtype, the potential side effects mediated through other opioid receptor types may be minimized or eliminated.

Some progress has been made in the development of highly selective opioid antagonists. For example, Portoghese et al. (U.S. Patent No. 4,816,586) disclose certain opiate analogs which possess high selectivity and potency at delta receptors. Minimal involvement was observed at mu and kappa opioid receptors. One of the highly selective analogs disclosed in U.S. Patent No. 4,816,586 has been named "naltrindole" or "NTI," and has the formula:



(NTI)

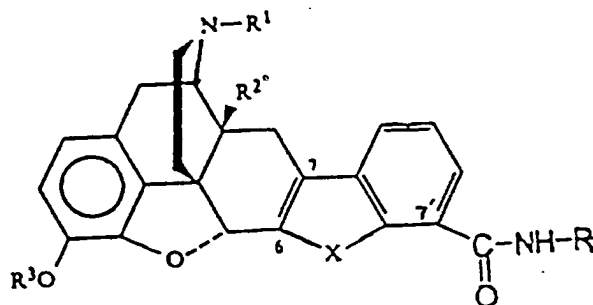
wherein X is NH. See P.S. Portoghese et al., J. Med. Chem., **31**, 281 (1988). Naltrindole has also recently been reported to have potent immunosuppressive activity. See, for example, K. Arakawa et al., Transplantation, **53**, 951 (1992).

Therefore, a continuing need exists for compounds which are opioid receptor-selective, i.e., which can act as agonists or antagonists with specificity at the delta, mu or kappa opioid receptor, or at one of the subtypes of these receptors.

Summary of the Invention

The present invention is directed to biologically active compounds of formula (I):

5



(I)

wherein R¹ is (C₁-C₅)alkyl, C₃-C₆(cycloalkyl)alkyl, C₅-C₇(cycloalkenyl)alkyl, (C₆-C₁₂)aryl, (C₆-C₁₂)aralkyl, *trans*(C₄-C₅)alkenyl, allyl or furan-2-ylalkyl, R² is
 10 H, OH or O₂C(C₁-C₅)alkyl; R³ is H, (C₆-C₁₀)aralkyl, (C₁-C₅)alkyl or (C₁-C₅)alkylCO; X is O, S or NY, wherein Y is H, benzyl or (C₁-C₅)alkyl; R is CH(Z)CO₂Y wherein Z is H, CH₂CO₂Y, or (CH₂)_nN(R⁴)(R⁵) wherein n is 1-5, preferably 3-4, and R⁴ and R⁵ are individually H, (C₁-C₄)alkyl, phenyl, (C=NH)NH₂, benzyloxycarbonyl or (C=NH)NHNO₂; preferably one or both of
 15 R⁴ and R⁵ are H; and the pharmaceutically acceptable salts thereof.

Using peptide antagonists of known binding selectivity as standards, it was found that the compounds of the invention are selective antagonists at delta opioid receptors, and that some are specific for the δ₁ or δ₂ subset of delta opioid receptors. All of the compounds preferably exhibit
 20 little or no binding at delta or mu receptors. Furthermore, compounds of the formula I were found to exhibit poor penetration into the central nervous system in *in vivo* animal models, while maintaining substantial peripheral effects. Thus, the present invention also provides a method for blocking delta opioid receptors in mammalian tissue or cells comprising said receptors, by
 25 contacting and complexing said receptors *in vivo* or *in vitro* with an effective amount of a compound of formula I, preferably in combination with a pharmaceutically acceptable vehicle. Thus, the compounds of formula I can be used as pharmacological and biochemical probes of opiate receptor structure and function, e.g., to measure the selectivity of other known or
 30 suspected opioid receptor antagonists or agonists at delta receptors. Procedures for such selectivity evaluations are disclosed hereinbelow, and in U.S. Pat. No. 4,816,586. Such tissue preferably includes tissue of the gut, the

cardiovascular system, the lung, the kidney, reproductive tract tissue and the like. Circulating cells comprising delta opioid receptors include lymphocytes, and delta receptor agonists and antagonists, have also been implicated in immunoregulatory responses related to T cell function, such as
5 immunosuppression.

Therefore, the compounds of formula I which exhibit delta receptor antagonist activity may also be therapeutically useful in conditions where selective blockage of delta receptors of non-CNS tissue or cells, is desired. Such conditions can include aspects of immunoregulation associated
10 with pathological stimulation of the immune system, as in autoimmune disorders, and in immunosuppression associated with certain viral infections, or as induced during organ transplantation, as well as a variety of other physiological activities that may be mediated through delta receptors. See, for example, Portoghese et al. U.S. Pat. No. 08/149,039 filed November 8, 1993,
15 and references cited therein. Also, certain of the compounds of formula I are useful as intermediates for the formation of other bioactive compounds within the scope of formula I. These include benzyl esters of carboxylic acids and nitrated or benzyloxycarbonyl-protected amines that are a part of R.

The alkyl moiety present in the R¹ group which links the
20 cycloalkyl, cycloalkenyl, aryl, or furan-2-yl moiety to the basic nitrogen atom in the compounds of formula I is a lower(alkyl) group, preferably -(CH₂)_n-, wherein n is about 1-5, most preferably n is 1, e.g., R¹ is C₃-C₆(cycloalkyl)methyl, C₅-C₇(cycloalkenyl)methyl, arylmethyl or furan-2-yl-methyl. Preferred aryl moieties include (C₆-C₁₀)aryl, i.e., phenyl, benzyl,
25 tolyl, naphthyl, anisyl and the like.

In structure I, a bond designated by a wedged or darkened line indicates one extending above the plane of the R³O-substituted phenyl ring. A bond designated by a broken line indicates one extending below the plane of the phenyl ring.

30 Preferred compounds of the formula I are those wherein R¹ is (C₁-C₅)alkyl, C₃-C₆(cycloalkyl)alkyl or C₅-C₇(cycloalkyl)methyl, and most preferably wherein R¹ is cyclopropylmethyl. R² is preferably OH or OAc

(O₂CCH₃), and R³ preferably is H. Preferably, X is NH, NHBz or NCH₃, most preferably NH. Preferably, R is CH(Z)CO₂H and Z is H, (CH₂)₃₋₄NHR⁴ wherein R⁴ is H or (C=NH)NH₂.

The ability of morphinan derivatives to cross the "blood-brain barrier" and to affect the central nervous system (CNS) is often far superior to that of peptide opioid antagonists. For example, as disclosed in U.S. patent application Serial No. 07/750,109, filed August 26, 1991, both NTI and its benzofuran analog, NTB were found to produce unexpectedly prolonged suppression of ethanol drinking in rats that were selectively bred for high voluntary ethanol drinking, as compared to peptidyl delta-opioid receptor antagonists. However, while the present compounds are also morphinan derivatives, the R group would be expected to be highly ionized, (in the ionic or zwitterionic form) at physiological pHs encountered in the mammalian gut. Thus, as discussed below, the access of these compounds to delta receptors in the CNS is restricted over previously known morphinans, including other NTI derivatives.

Processes of preparing the compounds of formula I are also aspects of the invention, as described hereinbelow, as are the novel intermediates employed in the syntheses, including compound 7, and its salts and esters.

Brief Description of the Drawings

Figure 1 is a schematic depiction of the synthesis of certain compounds of formula I.

25

Detailed Description of the Invention

Preparation of the compounds of formula I, which are formally N-substituted 7'-aminocarbonyl derivatives of NTI and its analogs, can be accomplished through amidation of a 7'-carboxyl group. Thus, as shown in Figure 1, the key intermediate employed in the synthesis of target compounds 2-6 was 7'-carboxynaltrindole (7). Compound 7 was obtained via the Fischer indole synthesis which involved refluxing equivalent amounts of naltrexone

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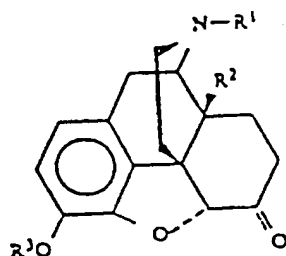
and 2-hydrazinobenzoic acid in glacial acetic acid. See. B. Robinson, The Fischer Indole Synthesis, Wiley-Interscience, NY (1982). The nmr spectrum of 7 possessed the characteristic downfield absorption of H-5 due to deshielding by the indole moiety. The coupling of 7 with suitably protected amino acids using the Bop reagent, afforded the corresponding intermediates 8a-e. Catalytic hydrogenation of these intermediates gave target compounds 2-6.

Compounds of formula I wherein X is O, S or NY can be prepared from intermediates analogous to 7 or 8 wherein NH has been replaced by O, S or NY.

The structures, common names and Merck Index reference numbers of representative 4,5-epoxy-6-ketomorphinan starting materials III, which can be used in place of naltrexone in the reaction scheme of Figure 1, are summarized in Table I, below.

Table I

5



(III)

10

	R^1	R^2	R^3	Common Name	Merck No. ²
	$CH_2CH(CH_2)_2$	OH	H	naltrexone	6209
	CH_3	OH	H	oxymorphone	6837
15	CH_3	H	H	hydromorphone	4714
	CH_3	H	CH_3	hydrocodone	4687
	$CH_2CH(CH_2)_2$	H	H	----	---- ¹
	$CH_2CH=CH_2$	OH	H	naloxone	6208
20	CH_3	OH	CH_3	oxycodone	6827

20

¹ Preparation: M. Gates et al., *J. Med. Chem.*, 7, 127 (1964).² The Merck Index, W. Windholz, ed., Merck & Co., Rahway, NJ (10th ed. 1983).

25

Other starting materials of the general formula III can be prepared by synthetic methods which are well known in the art of organic chemistry. For example, compounds wherein R^1 is H and R^3 is a suitable protecting group, and wherein the 6-keto group has also been protected, can be prepared from the compounds of formula III. These intermediates can be N-alkylated and deprotected to yield compounds of formula I wherein R^1 is C_2-C_5 (alkyl), C_4-C_6 (cycloalkyl)alkyl, C_5-C_7 (cycloalkenyl)alkyl, aryl, aralkyl, trans- C_4-C_5 alkenyl or furan-2-ylalkyl, by the application of well-known reactions.

30

For example, the free hydroxyl groups of the compounds of formula III, e.g., $R^2=OH$ and/or $R^3=H$, can be protected by acid-labile groups such as tetrahydropyranyl, trimethylsilyl, 1-methoxy-isopropyl and the like as disclosed in Compendium of Organic Synthetic Methods, I.T. Harrison et al., eds., Wiley-Interscience, New York, NY (1971) at pages 124-131, (hereinafter "Compendium"). The protection of the 6-keto group of compounds or a thioketal group is disclosed in Compendium, at pages 449-453. Methods for the demethylation of N-methyl amines have been disclosed, for example, in Compendium at page 247, J. Amer. Chem. Soc., **89**, 1942 (1967) and J. Amer. Chem. Soc., **77**, 4079 (1955).

Procedures for the alkylation of secondary amines with alkyl or aryl halides under basic or neutral conditions are well known. For example, see Compendium at pages 242-245; Org. Synth., **43**, 45 (1963); J. Org. Chem., **27**, 3639 (1962) and J. Amer. Chem. Soc., **82**, 6163 (1960).

Compounds of formula III wherein R^2 is acyloxy and/or R^3 is acyl can be prepared by using the corresponding starting materials on Table I. For example, naltrexone can be diacylated by reacting it with the appropriate (C_1-C_5) alkyl anhydride for 10-18 hrs at 18-25°C. The resultant 3,14-diacylated compound can be converted to the 14-acylated compound by limited hydrolysis. The 3-acylated starting materials can be prepared by the short-term reaction of the compounds of Table I with the anhydride, e.g., for about 2-4 hours. The 3-acylated product can be separated from the 3,14-diacylated product by chromatography.

The acid salts of compounds of formula I wherein $R^3=H$, can be converted into the corresponding (C_1-C_5) alkoxy derivatives [$R^3=(C_1-C_5)alkyl$] by dissolving the starting material in DMF and adding an excess of the appropriate (C_1-C_5) alkyl iodide and an amine such as diisopropylethylamine. The reaction can be conducted at an elevated temperature for about 4-10 hours. The final product can be purified by column chromatography.

The invention also comprises the pharmaceutically acceptable salts of the biologically active compounds of formula I together with a

pharmaceutically acceptable carrier for administration in effective, non-toxic dose form. Pharmaceutically acceptable amine salts may be salts of organic acids, such as acetic, citric, lactic, malic, tartaric, p-toluene sulfonic acid, methane sulfonic acid, and the like as well as salts of pharmaceutically acceptable mineral acids such as phosphoric, hydrochloric or sulfuric acid, and the like. Physiologically acceptable carboxylic acid salts include alkali metal carboxylates and quaternary ammonium salts. These physiologically acceptable salts are prepared by methods known in the art, e.g., by dissolving the free amine bases with an excess of the acid in aqueous alcohol, or neutralizing a free carboxylic acid with an alkali metal base such as a hydroxide; or with an amine.

In the clinical practice of the present method, the compounds of the present invention will normally be administered orally or parenterally, as by injection or infusion, in the form of a pharmaceutical unit dosage form comprising the active ingredient in combination with a pharmaceutically acceptable carrier, which may be solid, semi-solid or liquid diluent or an ingestible capsule or tablet. The compound or its salt may also be used without carrier material. As examples of pharmaceutical carriers may be mentioned tablets, intravenous solutions, suspensions, controlled-release devices such as patches or implants, microcapsules, liposomes and the like. Usually, the active substance will comprise between about 0.05 and 99%, or between 0.1 and 95% by weight of the resulting pharmaceutical unit dosage form, for example, between about 0.5 and 20% of preparation intended for injection or infusion and between 0.1 and 50% of preparation, such as tablets or capsules, intended for oral administration.

The invention will be further described by reference to the following detailed examples, wherein melting points were taken using a Thomas-Hoover Melting Point apparatus in open capillary tubes, and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona, and are within $\pm 0.4\%$ of the theoretical values. IR spectra were obtained on a Perkin-Elmer 281 infrared spectrometer and peak positions are expressed in cm^{-1} . NMR spectra were recorded at ambient temperature on

- GE-300 MHz and Bruker AC-200 MHz, and chemical shifts are reported as δ values (ppm) relative to TMS. Mass spectra were obtained on a VG 7070E-HF instrument. All TLC data were determined with E. Merck Art, 5554 DC-Alufolien Kieselgel 60 F₂₅₄. Column chromatography was carried out on E.
- 5 Merck silica gel 60 (230-400) mesh). The eluents used during column chromatography and reverse phase preparative HPLC, CHCl₃-MeOH-NH₄OH and MeOH-H₂O-CH₃CN, are denoted by CMA and MWA respectively. Dimethylformamide was distilled over calcium hydride. All other solvents and reagents were used without any further purifications unless specified.
- 10 Naltrexone hydrochloride was supplied by Mallinckrodt.

Example 1.

- 7'-Carboxy-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan hydrochloride (7).** Naltrexone
- 15 hydrochloride (2.0 g, 5.3 mmol) and 2-hydrazinobenzoic acid (1.2 eq, 1.1 g) were dissolved in glacial acetic acid (75 mL). The reaction was refluxed for 6 hrs. The solvent was evaporated under reduced pressure and the residue dissolved in a minimum amount of MeOH. The crude product was purified on column chromatography (silica gel) with CMA 95:5:0.5. Upon evaporation
- 20 of solvents under reduced pressure, the product was isolated as a solid which was recrystallized from methanol/ether. to afford 1.2 g of 7 (56%) : mp > 230°C; ¹H NMR (300 MHz, DMSO-d₆), δ 10.95 (d, 1H, COOH), 9.03 (s, 1H, PhOH), 7.75 (d, 1H, J = 7.50 Hz, Ph), 7.61 (d, 1H, J = 7.50 Hz, Ph), 7.04 (t, 1H, J = 7.20 Hz, Ph), 6.45 (m, 2H, Ph), 5.63 (s, 1H, H₅), 3.15 (m, 3H, H₉, H₁₀), 2.90-2.60 (m, 8H, H₈ H₁₈ H₁₆ H₁₅), 1.62 (m, 1H, H₁₅), 0.58 (m, 1H, H₁₉), 0.51 (m, 2H, H₂₀, H₂₁), 0.42 (m, 2H, H₂₀, H₂₁); ¹³C NMR (50 MHz, DMSO-d₆) δ 168.35, 143.11, 140.12, 131.04, 130.23, 127.70, 124.70, 123.53, 123.14, 118.57, 118.22, 117.28, 109.80, 83.39, 72.23, 61.40, 57.92, 46.78, 44.30, 29.97, 28.74, 23.10, 7.82, 4.41, 3.12; IR (KBr pellet) 3200, 1677 cm⁻¹; HRMS (FAB) 459 (M+H⁺), calcd. 459.1919, obsvd 459.1911; Anal. (C₂₇H₂₆O₅N₂•H₂O•HCl) C, H, N.
- 30

Example 2.**7'-Carboxamidoglycine-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan benzyl ester (8a).**

Carboxylic acid 7 (365 mg, 0.7 mmol) was dissolved in a mixture of
5 DMF/CH₂Cl₂ (40 mL) (1:1), in the presence of Et₃N (5.0 eq, 483 μ L),
benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate
(Bop reagent, Aldrich) (1.5 eq, 460 mg), and benzyl glycinate p-
toluenesulfonate salt, (1.0 eq, 257 mg). The reaction mixture was extracted
with ethyl acetate and the organic layer was washed with saturated NaHCO₃,
10 10% citric acid, and brine. The organic layer was dried over MgSO₄ and the
solvent removed *in vacuo*. The crude product was eluted on a silica gel
column with CMA (98:2:0.5) and purified further on preparative TLC (silica
gel, 1 mm) with CMA (98:2:0.5). The desired product was isolated as an oil
(150 mg, 36%) which was crystallized from chloroform/hexanes: mp: 118-
15 121°C; ¹H NMR (300 MHz, CDCl₃) δ 10.16 (s, 1H, PhOH), 7.52 (d, 1H, J =
7.50 Hz, Ph), 7.37 (m, 4H, Ph), 6.94 (d, 1H, H = 7.20 Hz, Ph), 6.89 (m, 1H),
6.64 (d, 1H, J = 8.40 Hz, H₂), 6.55 (d, 1H, J = 8.50 Hz, H₁), 5.64 (s, 1H, H₅),
5.24 (s, 2H, COOBn), 4.29 (d, 2H, J = 4.80 Hz, CH₂NH), 3.37 (d, 1H, J =
6.00 Hz, H₉), 3.10 (d, 1H, J = 18.30 Hz, H₁₀), 2.83-2.59 (m, 6H, H₁₆ H₁₈ H₁₀
20 NH), 2.45-2.29 (m, 3H, H₁₅ H₈), 1.80 (d, 1H, H = 11.10 Hz, H₁₅), 0.87 (m,
1H, H₁₉), 0.55 (m, 2H, H₂₁, H₂₀), 0.16 (m, 2H, H₂₀, H₂₁); ¹³C NMR (75 MHz,
CDCl₃) δ 171.43, 168.78, 163.45, 143.99, 140.22, 136.65, 135.89, 131.37,
130.92, 129.28, 129.25, 129.14, 129.11, 129.04, 128.57, 125.28, 123.65,
121.08, 119.48, 118.62, 118.12, 115.41, 111.35, 85.55, 73.49, 67.91, 62.89,
25 60.09, 48.66, 44.34, 42.18, 37.43, 37.19, 32.06, 29.32, 23.83, 10.09, 4.78.
4.42; HRMS (FAB) 606 (M+H⁺), calcd 606.2604, obsvd 606.2625: Anal.
(C₃₆H₃₅O₆N₃), C, N, N.

Example 3.**7'-Carboxamido-L-aspartate-17-(Cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan dibenzylester (8b).**

Carboxylic acid 7 (426 mg, 0.9 mmol) was dissolved in a mixture of

DMF/CH₂Cl₂ (40 mL) (1:1), in the presence of Et₃N (6.0 eq, 720 µL), Bop reagent (1.5 eq, 571 mg), and dibenzyl aspartate p-toluenesulfonate salt. The workup and purification were carried out as described for compound 8a. The desired product was isolated in 30% yield (187 mg, 0.2 mmol) and

5 crystallized from chloroform/hexanes: mp: 115-118°C; ¹H NMR (300 MHz, CDCl₃) δ 10.06 (s, 1H, OH), 7.59 (d, 1H, J = 7.50 Hz, Ph), 7.32 (m, 7H, Ph), 7.13 (d, 11H, J = 7.50 Hz, Ph), 6.85 (d, 1H, J = 7.20 Hz, Ph), 6.76 (d, 1H, J = 8.40 Hz, H₂), 6.65 (d, 1H, J = 8.50 Hz, H₁), 6.13 (m, 1H, NH), 5.77 (s, 1H, H₅), 5.21 (m, 2H, CH₂Ph), 5.13 (m, 2H, CH₂Ph), 5.09 (m, 1H, CHN), 3.38 (d, 10 2H, J = 4.80 Hz, CH₂COOBn), 3.26 (d, 1H, J = 5.10 Hz, H₉), 3.21 (d, 1H, J = 19.50 Hz, H₁₀), 2.89-2.71 (m, 3H), 2.57-2.33 (m, 5H), 1.84 (d, 1H, J = 9.90 Hz, H₁₅), 0.87 (m, 1H, H₉), 0.55 (m, 2H, H₂₀, H₂₁), 0.17 (m, 2H, H₂₀, H₂₁); ¹³C NMR (75 MHz, CDCl₃) δ 172.66, 172.26, 168.02, 143.81, 139.59, 136.34, 135.94, 135.58, 131.30, 129.30, 129.25, 129.11, 128.09, 128.02, 123.47, 15 121.48, 119.70, 118.09, 118.04, 117.85, 115.28, 115.25, 111.89, 86.31, 73.55, 68.61, 67.85, 62.84, 60.11, 49.83, 48.83, 44.37, 37.33, 32.44, 29.33, 23.83, 10.05, 4.78, 4.41; HRMS (FAB) M+H⁺, calcd 754.3128, obsvd 754.3138.

Example 4.

20 7'-(Carboxamido-[N(δ)-carbobenzyloxy]-L-ornithinate)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7,2',3'-indolomorphinan benzyl ester (8c). Carboxylic acid 7 (426 mg, 0.8 mmol) was dissolved in a mixture of DMF/CH₂Cl₂ 1:1 (30 mL), at room temperature, in the presence of Bop reagent (1.5 eq, 536 mg), Et₃N (5.0 eq, 564 µL) and

25 benzyl N(δ)-Cbz-L-ornithinate trifluoroacetate salt (380 mg, 0.8 mmol). The reaction mixture was stirred overnight. The workup and purification were carried out as described for compound 8a. The desired product was isolated as an oil 56% yield (362 mg): ¹H NMR (300 MHz, CDCl₃) δ 10.09 (broad s, 1H, PhOH), 7.51 (d, 1H, J = 8.70 Hz, Ph), 7.35 (m, 10H), 7.10 (d, 1H, J = 8.30 Hz, Ph), 6.88 (m, 1H, Ph), 6.61 (d, 1H, J = 8.40 Hz, H₂), 6.54 (d, 1H, J = 8.40 Hz, H₁), 5.57 (s, 1H, H₅), 5.20 (m, 2H, COOBn), 5.06 (m, 2H, Cbz), 30 4.90 (m, 1H, CHCOOBn), 3.34 (d, 1H, J = 6.30 Hz, H₉), 3.13 (m, 3H, H₁₀ -

CH₂NHCbz), 2.81-2.57 (m, 3H, H₁₀, H₁₆), 2.45-2.32 (m, 4H, H₈, H₁₈), 1.76 (m, 6H, CH₂'s H₁₅), 0.90 (m, 1H, H₁₉), 0.57 (m, 2H, H₂₀, H₂₁), 0.17 (m, 2H, H₂₀, H₂₁); ¹³C NMR (75 MHz, CDCl₃) δ 172.56, 167.91, 156.87, 143.24, 139.55, 136.18, 139.55, 136.68, 136.18, 135.32, 130.67, 130.40, 128.64, 128.45, 128.30, 128.15, 127.98, 124.67, 123.29, 120.58, 118.84, 118.04, 117.50, 115.12, 110.99, 84.73, 72.81, 67.28, 66.60, 62.32, 59.47, 52.41, 48.06, 43.70, 40.48, 31.51, 29.52, 28.70, 26.21, 22.46, 9.47, 4.12, 3.81; NRMS (FAB) 797 (M+H⁺), calcd 797.3550, obsvd 797.3547; IR (neat) 3300, 1719, 1703, 1613 cm⁻¹; Anal. (C₄₇H₄₈O₈N₄) C, H, N.

10

Example 5.

7'-(Carboxamido-N(ω,ω')nitro-L-argininate) 17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7,2',3'-indolomorphinan benzyl ester hydrochloride (8d). Carboxylic acid 7 (350 mg, 0.7 mmol) was dissolved in CH₂Cl₂/DMF (1:1) (50 mL), with Bop reagent (1.5 eq, 440 mg), Et₃N (5.0 eq, 463 mL), and benzyl N(α)-amino-N(ω,ω')-nitro-L-arginate trifluoroacetate salt (1.1 eq, 309 mg). The workup and purification steps were similar to those described for compound 8a. The desired product was isolated as a solid in 7% yield (33 mg); mp > 240°C; ¹H NMR (300 MHz, CDCl₃) δ 10.01 (broad s, 1H, PhOH), 8.80 (broad s, 1H, OH), 7.63 (m, 1H, Ph), 7.47 (m, 6H, Ph), 7.05 (m, 1H, H₅¹), 6.68 (d, 1H, J = 7.50 Hz, H₂), 6.55 (d, 1H, J = 7.50 Hz, H₁), 5.67 (s, 1H, H₃), 5.18 (broad m, 2H, COOBn), 5.04 (m, 1H, CHCOOBn), 3.34 (d, 1H, J = 6.30 Hz, H₉), 3.19 (m, 3H, H₁₀ -CH₂NH), 2.88-2.60 (m, 5H), 2.45-2.32 (m, 4H), 1.81 (m, 6H, CH₂'s H₁₅), 0.89 (m, 1H, H₁₉), 0.58 (m, 2H, H₂₁, H₂₀), 0.17 (m, 2H, H₂₀, H₂₁); ¹³C NMR (75 MHz, CDCl₃) δ 169.14, 167.04, 143.78, 140.22, 131.16, 129.07, 128.95, 128.80, 128.75, 125.10, 123.69, 121.59, 119.38, 118.83, 117.67, 111.05, 84.97, 73.58, 67.75, 62.71, 125.39, 123.18, 121.87, 119.37, 118.96, 117.99, 117.91, 111.17, 84.78, 80.25, 73.32, 62.61, 59.69, 50.46, 48.35, 44.41, 42.36, 32.26, 29.74, 28.79, 23.71, 22.57, 10.32, 4.99, 4.52; HMRS (FAB) 573 (M⁺) cation, calcd 573.2713, obsvd 573.2711; Anal. (C₃₂H₃₇O₆N₄·HCl) C, H, N: Calcd H, 6.08. Found H, 6.59.

Example 6.**7'-(Carboxamido-[N(ε)-Carbobenzyloxy]-L-lysinate) 17-**

(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7,2',3'-indolomorphinan benzyl ester (8e). Carboxylic acid 7 (385 mg, 0.93 mmol)

- 5 was dissolved in a mixture of DMF/CH₂Cl₂ (40 mL (1:1) (50 mL), in the presence of Et₃N (5.0 eq, 509 μL), Bop reagent (1.5 eq, 484 mg), and benzyl N(ε)-Cbz-L-lysinate trifluoroacetate salt (1.0 eq, 353 mg). The reaction was stirred overnight at room temperature. The workup and purification procedure was similar to that described for compound 8a. The desired product was
- 10 isolated as an oil in 30% yield (170 mg); ¹H NMR (300 MHz, CDCl₃) δ 10.12 (s, PhOH), 7.54 (d, 1H, J = 8.70 Hz, Ph), 7.43 (d, 1H, J = 7.20 Hz, Ph), 7.33 (m, 7H, Ph), 7.00 (m, 2H, Ph), 6.60 (d, 2H, J = 8.00 Hz, H₂), 6.53 (d, 2H, J = 8.00 Hz, H₁), 5.56 (s, 1H, H₃), 5.23 (m, 2H, COOBn), 5.04 (s, 2H, Cbz), 4.92 (m, 1H, CHCOOBn), 3.32 (d, 1H, J = 6.20 Hz, H₉), 3.09 (m, 3H,
- 15 H₁₀, CH₂NHCbz), 2.90-2.60 (bm, 3H, H₁₀, H₁₆), 2.50-2.30 (bm, 5H, H₁₆, H₈, H₁₀, H₁₈), 1.85 (bm, 3H, H₈ CH₂), 1.40 (bm, 6H, H₁, CH₂'s), 0.90 (m, 1H, H₁₉), 0.58 (m, 2H, H₂₀ H₂₁), 0.17 (m, 2H, H₂₀ H₂₁); ¹³C NMR (75 MHz, CDCl₃) δ 172.67, 167.83, 156.88, 143.24, 139.52, 136.62, 136.38, 135.57, 130.64, 130.54, 128.65, 128.45, 128.35, 127.99, 124.69, 123.32, 120.42,
- 20 118.80, 118.13, 117.40, 115.32, 110.95, 84.75, 72.75, 67.24, 66.62, 62.33, 59.48, 52.42, 43.71, 40.41, 31.89, 31.45, 29.25, 28.73, 23.14, 22.45, 9.46, 4.10, 3.81; HRMS (FAB) 811 (M+H⁺), calcd 811.3706, obsvd 811.3716; IR (neat) 3200, 1743, 1721, 1637 cm⁻¹.

25 **Example 7.**

7'-(Carboxamidoglycine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7,2',3'-indolomorphinan hydrochloride (2).

- Intermediate 8a (100 mg, 0.16 mmol) was dissolved in methanol (10 mL) in the presence of a catalytic amount (10%) of Pd-on-carbon and 2N HCl (1.5
- 30 eq, 12 μL). The hydrogenation reaction was run at atmospheric pressure for 1 hr. The mixture was filtered over celite and the celite washed several times with MeOH and the mixture was concentrated under reduced pressure. Slow

addition of EtOAc concentrate led to the precipitation of a solid which was purified by HPLC using a C₁₈ reverse phase column (silica gel) with MWA 40:30:30 plus a few drops of NH₄OH (Rt 50 mn). The crude product was purified by reverse phase HPLC (MWA 6:3:1), and the desired hydrochloride salt (Rt 11.87 mn) was isolated in 75% yield (68 mg): mp > 240°C; Cl₂/DMF (1:1) (50 mL), with Bop reagent (1.5 eq, 440 mg), Et₃N (5.0 eq, 463 mL), and benzyl N(α)-amino-N(ω,ω')-nitro-L-arginate trifluoroacetate salt (1.1 eq, 309 mg). The workup and purification steps were similar to those described for compound 8a. The desired product was isolated as a solid in 7% yield (33 mg); mp > 240°C; ¹H NMR (300 MHz, methanol-d₄) δ 10.76 (bs, 1H), 7.61 (m, 2H, H₆¹, H₄¹), 7.06 (t, 1H, J = 7.35 Hz, H₅¹), 6.64 (m, 2H, H₁, H₂), 5.75 (s, 1H, H₃), 4.15 (d, 2H, J = 6.20 Hz, CH₂N), 3.47-3.27 (m, 2H, H₉, H₁₀), 3.18-2.86 (m, 4H, H₁₈, H₁₀, H₈), 2.77-2.67 (m, 2H, H₈, H₁₅), 1.94 (bd, 1H, J = 13.50 Hz, H₁₅), 1.14 (m, 1H, H₁₉), 0.78 (m, 2H, H₂₀, H₂₁), 0.52 (m, 2H, H₂₀, H₂₁); ¹³C NMR (75 MHz, methanol-d₄) δ 171.57, 169.58, 141.39, 130.79, 128.54, 123.23, 123.11, 123.00, 121.78, 121.75, 119.89, 118.89, 118.76, 109.01, 84.08, 72.80, 62.82, 58.01, 51.78, 41.32, 41.21, 29.43, 28.99, 24.22, 6.04, 5.44, 2.48; HRMS (FAB) 516 (M+H⁺), calcd 516.2134, obsvd 516.2134; Anal. (C₂₉H₃₀O₆N₃•HCl) C, H, N.

20

Example 8.

7'-(Carboxamido-L-aspartate)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7,2',3'-indolomorphinan hydrochloride (3).

Intermediate 8b (180 mg, 0.2 mmol) was dissolved in methanol (15 mL) in the presence of a catalytic amount (10%) of Pd-on-carbon and 6N HCl (2.0 eq, 0.5 μL). The hydrogenation and purification procedures are as described for compound 2. The crude product was purified by reverse phase HPLC (MWA 3:6:1), and the diacid (Rt 9.50 mn) was isolated in 90% yield (130 mg, 0.2 mmol): mp > 210°C (dec); ¹H NMR (300 MHz, DMSO-d₆) δ 11.04 (s, 1H, PhOH), 9.04 (broad s, 1H, OH), 8.38 (d, 1H, J = 7.20 Hz, NH), 7.58 (d, 1H, J = 7.50 Hz, Ph), 7.53 (d, 1H, J = 7.50 Hz, Ph), 7.00 (t, 1H, J = 7.50 Hz, Ph), 6.48 (s, 2H, H₂, H₁), 5.63 (s, 1H, H₃), 4.06 (m, 1H, CHCOOH), 3.45

30

(m, 1, H₉), 3.29 (d, 2H, J = 3.60 Hz, CH₂COOH), 3.23-3.11 (m, 3H), 3.04-2.61 (m, 5H), 1.93 (d, 1H, J = 11.10 Hz, H₁₅), 1.18 (m, 1H, H₁₉), 0.72 (m, 2H, H₂₀, H₂₁), 0.50 (m, 2H, H₂₀, H₂₁); ¹³C NMR (75 MHz, DMSO-d₆) δ 174.63, 173.68, 167.07, 144.16, 141.09, 136.21, 132.16, 128.70, 123.19, 121.89, 119.62, 119.12, 118.30, 118.01, 110.03, 104.55, 104.13, 84.54, 73.28, 64.05, 62.47, 59.09, 50.01, 47.93, 45.50, 29.80, 24.02, 5.42, 4.23, 1.21; HRMS (FAB) 574 (M+H⁺), calcd 574.2189, obsvd 574.2191; Anal. (C₃₁H₃₂O₈N₃•HCl) C, H, N.

10 **Example 9.**

7'-(Carboxamido L-ornithine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5α-epoxy-6,7,2',3'-indolomorphinan hydrochloride (4).

Intermediate 8c (233 mg, 0.3 mmol) was dissolved in MeOH (20 mL) with 1N HCl (3 drops, 1.0 eq) and a catalytic amount of 10% Pd-on-carbon. The hydrogenation and purification were similar to those described for 2. The product was isolated in 85% yield (155 mg): mp > 240°C; ¹H NMR (300 MHz, DMSO-d₆) δ 10.92 (s, PhOH), 8.70 (d, 1H, J = 7.20 Hz, Ph), 7.67 (broad s, 1H, Ph), 7.61 (d, 1H, J = 7.20 Hz, Ph), 7.50 (d, 1H, J = 7.50 Hz, Ph), 6.98 (t, 1H, J = 7.50 Hz, Ph), 6.45 (s, 2H, NH), 5.60 (s, 1H, H₅), 4.36 (m, 1H, CHCOOH), 3.20-3.00 (m, 4H, H₉, H₁₀, CH₂NH₂), 2.90-2.40 (m, 8H, H₈, H₁₀, H₁₈, H₁₆, H₁₅), 1.90-1.70 (m, 5H, H₁₅, CH₂'s), 0.85 (m, 1H, H₁₉), 0.46 (m, 2H, H₂₀, H₂₁), 0.12 (m, 2H, H₂₁, H₂₀); ¹³C NMR (75 MHz, methanol-d₄) δ 171.16, 167.70, 144.13, 140.82, 136.30, 132.16, 132.05, 128.69, 6.70 (d, 1H, J = 7.50 Hz, H₂), (d, 1H, J = 7.50 Hz, H₁), 5.63 (s, 1H, H₅), 4.33 (m, 1H, CHCOOH), 3.35-3.12 (m, 4H, H₉, H₁₀, CH₂NH), 2.80-2.62 (m, 5H, H₁₀, H₁₈, H₁₆), 2.45-2.32 (m, 3H, H₈, H₁₅), 1.81-1.60 (m, 5H, H₁₅, CH₂'s), 0.85 (m, 1H, H₁₉), 0.46 (m, 2H, H₂₀, H₂₁), 0.12 (m, 2H, H₂₁, H₂₀); IR (KBr) 3200, 1735, 1644 cm⁻¹; HRMS (FAB) 617 (M⁺), calcd 617.3087, obsvd 617.3087; Anal. (C₃₃H₃₉O₆N₆•HCl) C, H, N.

Example 10.

7'-(Carboxamido-L-arginine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan hydrochloride (5).

Intermediate 8d (33 mg, 0.04 mmol) was dissolved in MeOH (5 mL), with a
5 catalytic amount (10%) of Pd on carbon, and HCl (3.0 eq, 100 μ L). The
hydrogenation and purification steps were as described for compound 2. The
desired compound was isolated in 90% yield (20 mg); mp > 250°C (dec); ¹H
NMR (300 MHz, DMSO-d₆) δ 10.05 (bs, 1H, PhOH), 7.38 (m, 3H, Ph),
59.88, 44.26, 41.08, 32.00, 29.09, 23.52, 9.74, 4.39, 3.89; IR (KBr) 3500,
10 1735, 1637 cm⁻¹; HRMS (FAB) 750 (M+H⁺), calcd 750.3251, obsvd 750.3242.

Example 11.

7'-(N(α)-Carboxamido-L-lysine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan hydrochloride (6).

15 Intermediate 8e (170 mg, 0.2 mmol) was dissolved in MeOH (20 mL) in the
presence of a catalytic amount (10%) of Pd-on-carbon and 1N HCl (3.0 eq,
600 mL). The hydrogenation and purification were as described for
compound 2. The desired hydrochloride salt was isolated in 65% yield (82
mg); mp > 270°C (dec); ¹H NMR (300 MHz, methanol-d₄) δ 7.64 (d, 1H, J =
20 7.50 Hz, Ph), 7.57 (d, 1H, J = 7.50 Hz, Ph), 7.05 (t, 1H, J = 7.50 Hz, Ph),
6.61 (broad s, 2H, Ph), 5.73 (s, 1H, H₅), 4.16 (d, 1H, J = 4.80 Hz, CHCOOH),
3.32-3.15 (m, 4H, H₉, H₁₀ CH₂), 3.04-2.97 (m, 3H, H₁₀ H₁₈ H₁₆), 2.87-2.67 (m,
4H, H₁₈ H₁₆ H₈), 1.96 (m, 4H, H₁₅ CH₂), 1.71 (m, 2H, CH₂), 1.51 (m, 2H,
CH₂), 0.90 (m, 1H, H₁₉), 0.85 (m, 2H, H₂₁, H₂₀), 0.51 (m, 2H, H₂₀, H₂₁); ¹³C
25 NMR (75 MHz, methanol-d₄) δ 174.95, 168.23, 144.20, 141.66, 136.41,
131.92, 130.29, 128.70, 123.45, 122.77, 122.55, 120.04, 119.09, 118.88,
117.39, 109.49, 84.03, 73.21, 62.08, 60.87, 57.85, 53.59, 47.07, 39.44, 31.01,
29.82, 29.33, 27.62, 24.70, 24.02, 6.86, 6.31, 3.65; HRMS (FAB) 587 (M+),
calcd 587.2869, obsvd 587.2857; IR (KBr) 3300, 1728, 1637 cm⁻¹; Anal.
30 (C₃₃H₃₉O₆N₄•HCl) C, H, N.

Example 12.**Pharmacological Results****A. Smooth Muscle Preparations**

All target compounds were tested on the electrically stimulated guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations as described previously by H.P. Rang, *J. Pharmacol. Chemother.*, **22**, 356 (1965) and G. Henderson et al., *Brit J. Pharmacol.*, **46**, 746 (1972), respectively, as reported in P.S. Portoghese et al., *Life Sci.*, **36**, 801 (1986). Antagonists were incubated with the preparations for 15 min prior to testing.

Morphine (M), ethylketazocine (EK), and [D-Ala²,D-Leu⁵]enkephalin¹⁷ (DADLE) were employed as μ -, κ -, and δ -selective agonists, respectively. Morphine and EK were employed in the GPI, and DADLE was used in the MVD. The antagonist potencies mediated through δ opioid receptors were expressed as Ke values which were calculated from the equation, $Ke =$

[antagonist]/(IC₅₀ ratio - 1), where the IC₅₀ ratio represents the IC₅₀ of the agonist in the presence of the antagonist divided by the control IC₅₀ of the agonist in the same preparation.

As demonstrated by the data summarized on Table 2, compounds 2-6 were potent and selective δ opioid antagonists with Ke values ranging from 1-5 nM. None were more potent than NTI at δ receptors, but the δ -selectivities were equivalent to or higher than that of NTI. In contrast to the results reported by Dappen et al. in U.S. Pat. No. 5,354,863 and 5,225,417 for carboxy-substituted derivatives of NTI, the precursor 7 exhibited δ antagonist potency approaching that of NTI. Thus, compound 7 and the use of a compound of formula I wherein the NHR group is replaced by OH, to antagonize the activity of ~~delta~~ opioid receptor agonists are also within the scope of the invention.

Table 2. Antagonist Potencies of 7'-Substituted Amino Acid Conjugates of Naltrindole in the MVD and GPI Preparations

Compd ^a	DADLE(δ) ^b		M (μ) ^c		EK (κ) ^c		IC ₅₀ Selectivity Ratio
	IC ₅₀ ratio	K _e (nM) ^d	IC ₅₀ ratio	IC ₅₀ ratio	IC ₅₀ ratio	δ/μ	δ/κ
5	1 (NTI) ^e	459±104	0.2	11.2±1.8	1.3±0.2	41	353
	2	32±8	3.2	1.5±0.1	1.1±0.2	21	30
	3	89±20	1.1	1.3±0.5	2.1±0.6	67	43
	4	58±15	1.8	3.0±1	0.9±0.0	20	63
	5	31±5	3.3	1.3±0.6	1.2±0.5	24	27
10	6	20±4	5.2	1.0±0.4	1.1±0.0	20	20
	7	25±7 ^f	0.4	2.0±0.3	1.0±0.2	122 ^g	231 ^g

- ^aThe concentration of antagonist was 100 nM unless otherwise specified. ^b[D-Ala²,D-Leu⁵]enkephalin in the MVD preparation. ^cMorphine (M) or ethylketazocine (EK) in the GPI preparation. ^dDerived from the Schild relationship (Schild, H.O. *Pharmacol. Rev.*, **9**, 242 (1957)) and calculated from an average of at least three IC₅₀ ratio determinations by using $K_e = [\text{antagonist}]/(\text{IC}_{50} \text{ ratio} - 1)$. ^eData from V.L. Korlipara et al., *J. Med. Chem.*, **37**, 1882 (1994). ^fThe concentration of 7 in the MVD experiment was 10 nM. ^gA calculated IC₅₀ ratio (238) based on 100 nM of 7 was employed to calculate the selectivity ratio.

B. Receptor Affinity

- The opioid receptor affinities of the target compounds were determined on guinea pig membranes employing a modification of the method of Werling et al., *J. Pharmacol. Exp. Ther.*, **233**, 722 (1986). Binding to κ , μ , and putative " δ_1 " and " δ_2 " sites was evaluated by competition with

[³H]U69593, [³H][D-Ala²,MePhe⁴,Gly-ol⁶]enkephalin ([³H]DAMGO), [³H][D-Pen²-D-Pen³]enkephalin ([³H]DPDPE), and [³H][D-Ser²-Leu⁵]enkephalin-Thr⁶ ([³H]DSLET), respectively. As shown by the data on Table 3, each of compounds 2-6 and the 7'-carboxy starting material 7 possessed high affinity for δ_1 sites. The glycinate 2 nad precursor 7 possessed 2 and 6 times greater affinity for δ_1 sites than NTI, while other members (3-6) of the series had 5-10 fold less affinity than that of NTI.

All of the compounds were also highly selective, with K_i ratios substantially greater than that of NTI. It is noteworthy that the glycinate conjugate 2 possessed the highest selectivity ratios, with values of μ/δ_1 and κ/δ_1 in excess of 30,000. Interestingly, the glycine and ornithine conjugates (2 and 4) competed more effectively with [³H]DSLET than with [³H]DPDPE for δ sites by a factor of 8-10. The aspartate and lysine derivatives (3 and 6) exhibited a modest preference for [³H]DPDPE sites. However, as discussed below, there is no clear correlation between binding and a specific pharmacologic antagonist potency *in vitro* or *in vivo*.

Table 3. Binding of 7'-NTI Derivatives to Guinea Pig Brain Membranes

Compound	K_i (nM) ^a			K_i Selectivity Ratio		
	μ	κ	δ_1	δ_2	μ/δ_1	κ/δ_1
1 (NTI) ^b	3.8	332	0.03		127	11066
2	206	162	0.005	0.00066	38166	30037
3	368	893	0.14	0.41	2574	6246
4	136	57	0.18	0.015	756	320
5	333	188	0.16	0.083	2096	1181
6	219	41	0.30	0.75	719	134
7	24	42	0.013	0.016	1846	3246
						8.2
						0.3
						11.3
						1.9
						0.4
						0.8

^aThe geometric mean of K_i values from three replicate determinations for [³H]DAMGO, [³H]U69593, [³H]DPDPE, and [³H]DSLET, representing μ , κ , δ_1 and δ_2 sites, respectively.

^bData from P.S. Portoghese et al., *J. Med. Chem.*, **33**, 1714 (1990).

C. In Vivo Studies

Two of the more selective antagonist ligands (2, 3) and NTI (1) were evaluated in male Swiss-Webster mice using the tail-flick assay according to the methodology of F.C. Tulunay et al., J. Pharmacol. Exp. Ther., 190, 395 (1974). Mice were pretreated with the antagonist by the i.c.v. or i.v. route so that the peak antagonist activity coincided with the peak antinociceptive response of the selective agonists. The nonpeptide μ and κ agonists, morphine and *trans*-(\pm)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50488), were administered s.c. (von Voigklander, J. Pharmacol. Exp. Ther., 224, 7 (1983)); the peptide agonists (DPDPE and DSLET) were injected i.c.v.

As shown by the data presented in Table 4, when administered either i.v. or i.c.v., the glycine conjugate 2 effectively antagonized the antinociceptive effect of the δ_1 agonist, DPDPE. No significant antagonism of DSLET (δ_2), morphine (μ), or U50488 (κ) was observed. Similarly, the aspartate conjugate 3 selectively antagonized DPDPE.

**Table 4. Antagonist Profiles of Compounds 2 and 3 After
Intracerebroventricular (i.c.v.)
and Intravenous (i.v.) Administration in Mice**

Agonist	Pretreatment ^b		Control ED ₅₀ (95% C.I.) ^c	ED ₅₀ Ratio (95% C.I.) ^d
	Compound	Dose (Route of Injection)		
Morphine (μ)	2	20 pmol/kg (i.c.v.)	9.7 (8.6-10.9) μ mol/kg s.c.	0.9 (0.8 - 1.1)
	3	20 pmol/kg (i.c.v.)		1.0 (0.7 - 1.5)
U50,488H (κ)	2	20 pmol/kg (i.c.v.)	27.3 (8.5-52.8) μ mol/kg s.c.	0.8 (0.7 - 0.9)
	3	20 pmol/kg (i.c.v.)		0.7 (0.4 - 1.2)
DSLET (δ_2)	2	20 pmol/kg (i.c.v.)	0.7 (0.3-1.0) nmol, i.c.v.	1.1 (0.8 - 1.6)
	3	20 pmol/kg (i.c.v.)		0.5 (0.3 - 1.1)
DPDPE (δ_1)	2	20 pmol/kg (i.c.v.)	8.6 (6.5-11.1) nmol, i.c.v.	5.3 (3.6 - 7.7)*
	3	20 pmol/kg (i.c.v.)		2.6 (2.0 - 3.6)*
	2	0.9 μ mol/kg (i.v.)		2.7 (1.9 - 4.0)*
	3	0.8 μ mol/kg (i.v.)		2.3 (1.7 - 3.1)*
	1	0.4 nmol/kg (i.c.v.)		2.6 (1.9 - 3.9)*
	1	12.5 μ mol/kg (i.v.)		6.7 (4.6 - 10.0)*

^aPeak antinociceptive activity times of agonists were: morphine (30 min), U50,488H (20 min), DSLET (10 min) and DPDPE (20 min).

^bAntagonists were administered so that their peak activities coincided with the peak agonist activities. Pretreatment times were: 2 (30 min i.c.v., 90 min i.v.), 3 (20 min i.c.v., 30 min i.v.) and 1 (30 min i.c.v., 40 min i.v.).

^cAntinociceptive activities were determined by tail flick assay in mice.

^dED₅₀ with antagonist/control ED₅₀.

The doses required to produce equivalent antagonism i.c.v. and i.v. are listed in Table 5. These doses were determined by calculating the dose of antagonist required to double the ED₅₀ dose of DPDPE. The i.v./i.c.v. dose ratios of 2 and 3 were 15- and 7-times greater than that of NTI.

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Table 5. Dose Required to Produce an Equivalent Antagonist of DPDPE-induced Antinociception^a

Compound	i.c.v.	i.v.	Ratio (i.v./i.c.v.)
10 1	0.3 nmol/kg	2.19 μ mol/kg	7,300
2	4.7 pmol/kg	0.53 μ mol/kg	112,766
3	12.5 pmol/kg	0.62 μ mol/kg	49,600

^aDose required to double the ED₅₀ of DPDPE (antagonist dose/ED₅₀ ratio - 1).

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D. Discussion

The present invention provides polar δ -selective antagonists that are less accessible to the CNS after peripheral administration. Therefore, these compounds can provide useful tools to resolve the central from the peripheral effects mediated by δ opioid receptors.

The conjugates 2-6 and their precursor 7 were found to be potent δ opioid receptor antagonists in the mouse vas deferens preparation and they displayed little, if any, antagonism toward μ - and δ -selective agonists in the guinea pig ileum. In fact, the δ/μ and δ/κ IC₅₀ selectivity ratios of these ligands were equal to or greater than that of NTI.

Binding studies revealed that the K_i values of these antagonists were in the sub-nanomolar range for δ receptors. It is noteworthy that some of the conjugates (2, 4) appeared to have greater affinity for δ_2 relative to δ_1 putative binding sites, while others (3, 6) exhibited a modest preference for δ_1 over δ_2 sites. The fact that these binding selectivity ratios do not correlate

with the *in vivo* δ_1/δ_2 selectivity ratios of 2 and 3, as discussed below, suggests that the binding data may be of limited value to predict pharmacologic potency of antagonists in these studies.

The glycinate 2 and aspartate 3 conjugates were evaluated for their effectiveness as antinociceptive agents in mice by peripheral (i.v.) and central (i.c.v.) routes of administration. Both 2 and 3 were δ_1 selective in that they antagonized the δ_1 agonist, DPDPE, but not the δ_2 agonist, DSLET. It is not entirely clear why the binding selectivity of 2 and 3 differ from their pharmacologic selectivity, but one possibility is that accessibility to the δ_1 subtype *in vivo* might be favored by the presence of hydrophilic groups in these compounds. Thus, it is conceivable that the greater water solubility of the conjugates might facilitate access to tissue compartments in the CNS that contain δ_1 sites or prevent access to compartments that contain δ_2 sites. An alternate possibility is that identical δ receptors are located in different tissue compartments whose accessibility is governed by the polarity of the antagonist. Significantly, the *in vivo* δ_2 -selective antagonists, naltriben (NTB) and benzylnaltrindole, are considerably more lipophilic than 2 or 3. See Q. Jiang et al., J. Pharmacol. Exp., **257**, 1069 (1991) and V.L. Korlipara et al., J. Med. Chem., **37**, 1882 (1994).

As shown on Table 4, the i.v./i.c.v. dose ratios of 2 and 3 to produce equivalent antagonism of DPDPE-induced antinociception were very high ($> 49,000$) and, as expected, greater than that determined for NTI. This ratio was 15- and 7-fold greater than that of NTI for 2 and 3, respectively, which indicates that NTI penetrates the CNS more readily than the conjugates. It was surprising that the aspartate 3 has a lower i.v./i.c.v. dose ratio than the glycinate 2 in view of the greater polarity of the aspartate relative to the glycinate residue.

In summary, amino acid conjugates 2 and 3 are highly potent δ opioid antagonists when administered centrally, and many orders of magnitude less potent when delivered by the peripheral route. The *in vivo* δ_1 selectivity of 2 and 3 may be a function of the greater hydrophilic character imparted to the ligands by the amino acid residue. If, for example, this promotes greater

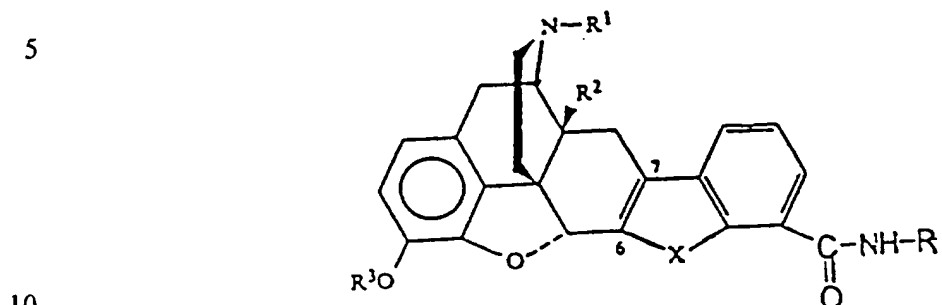
accessibility of the ligand to the δ_1 receptor subtype in neural tissues, it may suggest a new approach to the design of *in vivo* pharmacologically selective antagonist ligands for δ opioid receptor subtypes localized in the CNS. If the origin of the pharmacologic selectivity of NTI analogs for central δ opioid

5 receptor subtypes is in part related to selective access to tissue compartments rather than to binding selectivity, this would explain the lack of correlation between *in vivo* pharmacologic selectivity and binding selectivity. For this reason, the subtype selectivity of compounds such as 2 and 3 for antagonism of antinociception may not be the same for δ receptors at peripheral sites.

10 The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A compound of the formula:



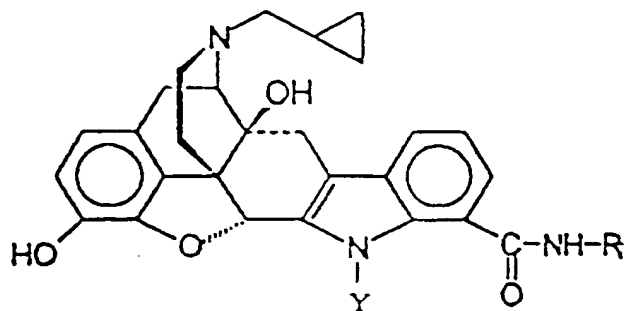
wherein R¹ is (C₁-C₅)alkyl, C₃-C₆(cycloalkyl)alkyl, C₅-C₇(cycloalkenyl)alkyl, (C₆-C₁₂)aryl, (C₆-C₁₂)aralkyl, trans(C₄-C₅)alkenyl, allyl or furan-2-ylalkyl, R² is H, OH or O₂C(C₁-C₅)alkyl; R³ is H, (C₆-C₁₀)aralkyl, (C₁-C₅)alkyl or (C₁-C₅)alkylCO; X is O, S or NY, wherein Y is H, benzyl or (C₁-C₅)alkyl; R is CH(Z)CO₂Y, wherein Z is H, CH₂CO₂Y or (CH₂)_nN(R⁴)(R⁵), wherein n is 1-5 and R⁴ and R⁵ are individually H, (C₁-C₄)alkyl, phenyl, (C=NH)NH₂, benzyloxycarbonyl or (C=NHNO₂); and the pharmaceutically acceptable salts thereof.

- 20 2. The compound of claim 1 wherein R¹ is C₃-C₆(cycloalkyl)-C₁-C₅(alkyl).
3. The compound of claim 2 wherein R¹ is C₃-C₆(cycloalkyl)-methyl.
- 25 4. The compound of claim 3 wherein R¹ is cyclopropylmethyl.
5. The compound of claim 1 wherein R² is OH or OAc.
6. The compound of claim 5 wherein R³ is H.
- 30 7. The compound of claim 1 wherein X is NH, N(benzyl) or NCH₃.

8. The compound of claim 1 wherein Y is H.
9. The compound of claim 8 wherein R⁴ and R⁵ are H.
10. The compound of claim 9 wherein R⁴ is H and R⁵ is (C=NH)NH₂.
11. The compound of claim 10 wherein n is 3-4.
12. A compound of the formula:

10

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wherein Y is H, CH₃ or benzyl; R is CH(Z)CO₂Y, wherein Z is H, CH₂CO₂Y or (CH₂)₃₋₄NHR⁴, wherein R⁴ is H or (C=NH)NH₂; and the pharmaceutically acceptable salts thereof.

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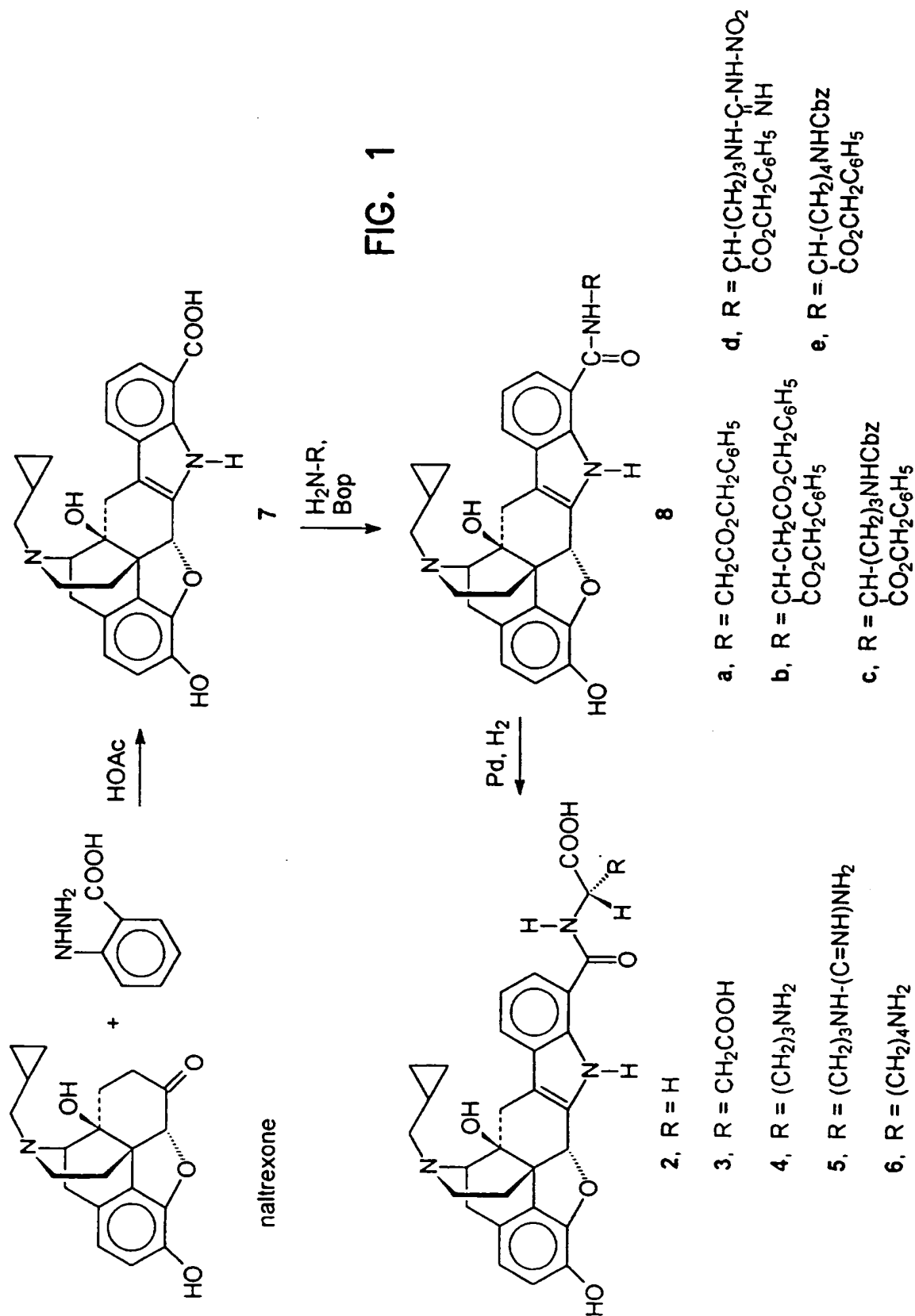
13. The compound of claim 12 wherein Y is H.
14. The compound of claim 12 wherein R⁴ is H.
15. The compound of claim 14 wherein R⁴ is (C=NH)NH₂.
16. The compound of claim 12 wherein Z is CH₂CO₂H.
17. 7'-(Carboxamidoglycine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan.

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18. 7'-(Carboxamido-L-aspartate)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan.
19. 7'-(Carboxamido L-ornithine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan hydrochloride.
20. 7'-(Carboxamido-L-arginine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan hydrochloride.
21. 7'-(N(α)-Carboxamido-L-lysine)-17-(cyclopropylmethyl)-6,7-dehydro-3,14-dihydroxy-4,5 α -epoxy-6,7,2',3'-indolomorphinan hydrochloride.
22. A method of antagonizing the action of a delta-opioid agonist at a delta opioid receptor comprising contacting a mammalian tissue or mammalian cell, wherein said tissue or cell incorporates delta-opioid receptors, with an amount of a compound of claim 1 effective to bind to said delta-opioid receptors.
23. The method of claim 22 wherein the tissue is gastrointestinal tissue.
24. The method of claim 22 wherein the cells are T-lymphocytes.
25. The method of claim 22 wherein the delta opioid receptors are δ_1 receptors.

FIG. 1



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/01171

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D491/20 C07D489/08 A61K31/485 //(C07D491/20,307:00,
221:00,209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 614 898 (TORAY INDUSTRIES) 14 September 1994 see claims 1,8-11	1,12,22
P,X	--- JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 3, 3 February 1995, WASHINGTON US, pages 402-407, XP002002656 P. S. PORTUGHESE ET AL: "7-Substituted amino acid conjugates of naltrindole. Hydrophilic groups as determinants of selective antagonism of delta1 opioid receptor-mediated antinociception in mice" see the whole document -----	1,12,22

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

9 May 1996

Date of mailing of the international search report

24 May 1996 (24.05.96)

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/01171

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-614898	14-09-94	AU-B- 4834193	26-04-94
		FI-A- 942499	27-07-94
		NO-A- 941977	29-07-94
		CA-A- 2124455	14-04-94
		CN-A- 1092425	21-09-94
		WO-A- 9407896	14-04-94

INTERNATIONAL SEARCH REPORT

ernational application No.

PCT/US 96/ 01171

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 22-25 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.